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Review

Gene therapy for heart failure

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ABSTRACT

Heart failure is a major public health problem throughout the world and it is likely that its prevalence will continue to grow over the next several decades. Despite advances in the treatment of heart failure, morbidity and mortality remain unacceptably high. Gene transfer therapy provides a novel strategy for targeting abnormalities in cardiac cells that adversely affect cardiac function. New vectors for gene delivery, mainly adeno-associated viruses (AAVs) that are preferentially taken up by cardiomyocytes, can result in sustained transgene expression. The cardiac isoform of sarco(endo)plasmic reticulum Ca^{2+}ATPase (SERCA2a) plays a major role in regulating calcium levels in cardiomyocytes. Abnormal calcium handling by the failing heart caused by a reduction in SERCA2a activity adversely affects both systolic and diastolic function. The Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease (CUPID) study was a Phase 2a double-blind, randomized, placebo-controlled, dose-finding study that was performed in patients with advanced heart failure due to systolic dysfunction. Eligible patients received AAV/SERCA2a or placebo by direct antegrade infusion into the coronary circulation. At the end of 12 months, patients receiving high-dose therapy (i.e. 1×10^{13} DNase Resistant Particles) had evidence of favorable changes in several clinically relevant domains compared to patients treated with placebo. There were no safety concerns at any dose of AAV/SERCA2a. Patients treated with AAV/SERCA2a exhibited a striking reduction in cardiovascular events that persisted through 36 months of follow-up compared to patients who received placebo. Transgene expression was detected in the myocardium of patients receiving AAV/SERCA2a gene therapy as long as 31 months after delivery. A second Phase 2b study, CUPID 2, designed to confirm this favorable effect on heart failure events, is currently underway with the results expected to be presented later in 2015. Additional studies using other vectors and targets are in planning or underway making gene transfer therapy one of the most exciting new approaches under development for treating heart failure.

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Introduction

Heart failure is a global public health problem of considerable magnitude with increases in prevalence noted in virtually every region of the world [1–6]. The aging of the population is the most important factor in this growth in the number of heart failure patients. Improved survival with other cardiovascular diseases for which heart failure is a late consequence, most notably myocardial infarction (MI), has also contributed to this pandemic as has the rapid rise in conditions such as obesity and diabetes that predispose to heart failure [7–9]. Although many effective therapies have been developed over the past 50 years, heart failure morbidity and mortality remain unacceptably high and new approaches for treating the increasing worldwide population of heart failure patients are needed.

Gene transfer therapy

Gene transfer therapy refers to the introduction of recombinant human genetic material to a patient in order to alter levels of a protein that will either directly or indirectly (e.g. through paracrine or systemic effects) affect organ function. Advances in cellular and molecular biology over the past several decades have offered insights into fundamental processes that lead to the development of heart failure [10]. These include abnormalities in adrenergic signaling, alterations in calcium handling, disturbances cardiomyocyte relaxation, activation of pathways that result in cell death and stimulation of pathways that lead to deposition of extracellular matrix within the myocardium. Changes in the levels or activity of molecules that play critical roles in these pathways have been identified and many of these abnormalities are potentially treatable by gene transfer therapy [11]. In addition, monogenetic disorders that are the cause of heart failure are also inviting targets for gene transfer therapy.

The conditions required for successful gene transfer therapy are dependent on the characteristics of the disease. Patients with chronic heart failure suffer from a condition that is organ wide. Even when the initiating cause of heart failure is a discrete event in both time and distribution (e.g. MI), progressive changes in the structure and function of spared myocardium due to increases in regional wall stress and the influence of neurohormonal systems that are activated in response to injury expands the area that is involved to encompass the entire heart. Thus, gene transfer to treat heart failure should target as much myocardium as possible. Furthermore, since heart failure is a chronic, progressive disease, therapy needs to be sustained over time in order for lasting benefits to be realized.

Gene delivery systems

Both non-viral and recombinant viral delivery systems for gene transfer therapy have been developed. Whereas non-viral systems are relatively easy to produce, allow incorporation of large size cassettes of recombinant genetic material and have low biosafety risk, they are used less often due to problems with transfection efficiency and intracellular degradation of the genetic material. Recombinant viral systems have become the preferred mode of delivery based on high efficiency and the capacity for long-term transgene expression. Some viral delivery systems are limited by their reduced capacity to package genetic material. Previous concerns about inconsistencies in bioavailability, purity, and biosafety, however, have been largely addressed and viral vectors have now been used to deliver genetic material to humans in numerous clinical trials.

Among the viral vectors used for gene therapy, adenovirus, adeno-associated virus (AAV) [12], and lentivirus vectors [13] have been most studied. AAVs have emerged as the preferred vector for gene delivery based on the fact that they are minimally immunogenic, have the capacity for long-term transgene expression, and have demonstrated an excellent biosafety record. In addition, several strains of vectors are highly cardiotropic so that problems associated with gene delivery to other organs throughout the body can be minimized [14]. An important limitation of using AAV vectors, however, is the presence of neutralizing antibodies due to prior infection in a substantial percentage of patients.

Gene transfer therapy for heart failure can be accomplished by either direct introduction into the heart or presentation to the myocardium via the circulation [15–17]. The former can be accomplished by direct injection into the epicardial surface of the heart. As this requires fairly wide exposure in order to reach all segments of the myocardium, it is most useful when carried out during open-heart surgery for another reason such as placement of a ventricular assist device (VAD). Endocardial delivery is also possible using catheter techniques that allow micropuncture and delivery of the reagent at multiple sites in the ventricle at standard depths. Delivery of genetic material through the circulation can be accomplished by either direct antegrade injection through the coronary arteries or retrograde introduction through the coronary sinus into the venous system of the heart. When delivered in the circulation, viral particles bind to surface receptors and are taken up into cardiac cells by endocytosis. The viral particles are then released into the cytoplasm where they are transported to the nucleus where the viral capsid is disassembled to release the genetic material. After double-stranded synthesis of the gene of interest is complete, transcription and translation begin leading to the production of the molecule of interest.

Targeting sarcoendoplasmic reticulum ATPase 2a to treat heart failure

Dysregulation of calcium handling in cardiomyocytes plays an important role in the contractile and relaxation abnormalities that are seen in the failing heart [18,19]. Calcium that is released into the cytosol from the sarcoplasmic reticulum during systole activates actin and myosin coupling that results in myofilament shortening and the production of force. Re-uptake of calcium during diastole governs the rate of relaxation. Control of calcium flux within cardiomyocytes is regulated by a variety of channels and enzymes including the L-type calcium channel, the sodium/calcium exchanger, the ryanodine receptor, and sarcoendoplasmic reticulum ATPase (SERCA) 2a activity.

The role of SERCA2a is of particular importance [20]. This enzyme is situated on the surface of the sarcoplasmic reticulum (SR) where it regulates the re-uptake of calcium from the cytosol into the SR during diastole. Not only does the reduction in cytosolic calcium concentration determine the relaxation but it also allows an adequate store of calcium to be available within the SR for release during the ensuing systole. Downregulation of SERCA2a has been demonstrated in both experimental animal models and human patients with heart failure [21–27]. In homogenates of human heart, even a modest decrement in SERCA2a protein reduces enzyme activity to the extent that there is a substantial increase in diastolic calcium concentration [28]. Abnormalities in calcium flux, relaxation, and contraction of cardiomyocytes from the failing heart are associated with reduced SERCA2a activity and they can be corrected by increasing SERCA2a activity by gene transfer therapy. Both small and large animal models of heart failure have been shown to respond favorably to treatment with gene transfer therapy using a viral vector to deliver the SERCA2a gene to the heart [29–33].

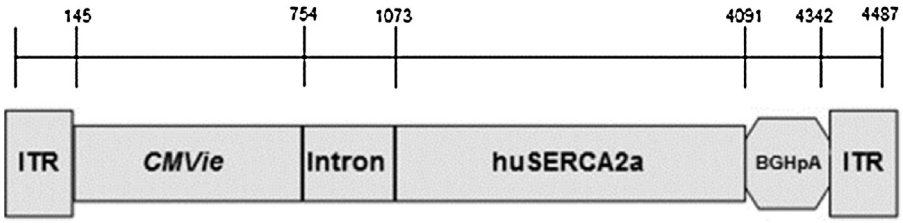

Fig. 1. Structure of the adeno-associated virus (AAV)1/sarco(endo)plasmic reticulum Ca^{2+}ATPase (SERCA2a) vector used in the Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease (CUPID) studies. The AAV1/SERCA2a vector only incorporates inverted terminal repeats (ITRs) from AAV2 serotype, resulting in <300 nucleotides of the wild-type AAV sequences in the vector genome. The single-stranded DNA is encapsulated in the AAV serotype 1 icosahedral capsid. The SERCA2a expression cassette contains the following components: the cytomegalovirus immediate early enhancer/promoter (CMVie) driving transcription of sequences including a hybrid intron from the commercial plasmid pCI, the huSERCA2a cDNA, and a bovine growth hormone polyadenylation signal (BGHpA). Reproduced with permission from Greenberg et al. [38].

Gene transfer therapy with SERCA2a in heart failure patients

The CUPID study used a modified Type 1 adenovirus, depicted in Fig. 1, to deliver the human cDNA for SERCA2a to the hearts of patients with advanced heart failure [34–36]. Entry criteria for the study included the presence of advanced New York Heart Association functional class III or IV symptoms of heart failure due to either ischemic or non-ischemic disease and an ejection fraction (EF) ≤0.35. Patients were required to be clinically stable on optimal medical therapy for at least 30 days prior to entry, to have impaired exercise capacity, and to have neutralizing antibody titers of <1:2.

Qualifying patients were randomized in a double-blinded fashion to receive either low, mid or high dose of AAV1/SERCA2a or placebo by direct antegrade intracoronary injection over a 10-min period. Patients were pre-treated with low-dose intravenous nitroglycerin whenever possible based on preliminary data suggesting that this strategy enhanced uptake into cardiomyocytes [37]. Patients were then actively followed over a 12-month period to determine the safety and efficacy of the treatment regimens and then for an additional 24 months to assess safety. The study used a novel predefined primary endpoint for efficacy that was based on concordant changes across multiple efficacy domains. The domains that were used, variables assessed, and criteria for what was considered a meaningful change in individual patients are outlined in Table 1.

Table 1
Domains and variables used to assess the effects of AAV1/SERCA2a treatment in CUPID.

Efficacy domain	Meaningful change
Symptomatic	
NYHA class	1 class
MLWHFQ	10 points
Functional	
6MWT	50 m
Peak VO_2	1.5 mL/kg/min
Biomarker	
NT-proBNP	35% or 300 pg/mL[a]
Remodeling	
Ejection fraction	3 or 5% absolute[b]
End-systolic volume	20 mL or 10%[a]

Reproduced with permission from Jessup et al. [35].
AAV, adeno-associated virus; NYHA, New York Heart Association; SERCA2a, sarco-endoplasmic reticulum Ca^{2+}ATPase; MLWHFQ, Minnesota Living With Heart Failure Questionnaire; 6MWT, 6-minute walk test; VO_2, maximal oxygen consumption; mL/kg/min, milliliters/kilogram/minute; NT-proBNP, N-terminal of the prohormone brain natriuretic peptide; pg/mL, picograms/milliliter; mL, milliliter.
[a] Whichever is greater.
[b] 3% for group, 5% for individual.

The baseline characteristics of the CUPID population are outlined in Table 2. Over the course of the study, there was no evidence of an increase in any treatment emergent adverse event in any of the groups that received gene transfer therapy nor were there significant differences between study groups in vital signs, blood chemistries, hematologic variables, electrocardiographic parameters, or arrhythmia burden. In contrast to patients in the placebo group who showed evidence of worsening in measures of heart failure severity across the domains, patients in the high-dose (1×10^{13} DRP) group demonstrated improvement or stabilization in all variables measured. These results are summarized in Fig. 2. Thus, for the high-dose group, the CUPID study met its pre-specified efficacy endpoint. Simulation of the potential outcomes of the CUPID study based on permutation test indicated a high level of confidence (>1:1000) that the results had not come about as a play of chance.

Clinical events were also followed in the CUPID patients and are depicted at the 1- and 3-year time points in Fig. 3. There was a striking reduction in cardiovascular events in the high-dose compared to the placebo cohort at the end of 12 months. Even after 36 months, the differences in event rate between the high-dose and placebo patients remained apparent. Hospitalization for heart failure was common, as would be expected in the advanced heart failure population included in CUPID. Treatment

Table 2
Description of patients enrolled in CUPID.

Characteristic	All subjects *N* = 39
Age, years, mean (SD)	60.5 (11.5)
Sex, *n*	34 male
Race, *n*	34 White
HF etiology, *n* (%)	
Ischemic cardiomyopathy	19 (48.7)
Idiopathic cardiomyopathy	14 (35.9)
Hypertensive cardiomyopathy	4 (10.3)
Other	3 (7.7)
6MWT, m, mean (SD)	343 (124)
VO_2 max, mL/kg/min, mean (SD)	13.9 (3.9)
LVEF, %, mean (SD)	25 (7)
LVESV, mL, mean (SD)	202 (91)
NYHA class III, *n* (%)	39 (100)
MLWHFQ, mean (SD)	46 (22)
NT-proBNP, pg/mL, mean (SD)	2932 (3028)

Reproduced with permission from Jessup et al. [35].
CUPID, Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease; SD, standard deviation; *n*, number; HF, heart failure; 6MWT, 6-minute walk test; VO_2 max, peak oxygen consumption; mL/kg/min, milliliters/kilogram/minute; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume; NYHA, New York Heart Association; MLWHFQ, Minnesota Living With Heart Failure Questionnaire; NT-proBNP, N-terminal prohormone brain natriuretic peptide.

Efficacy Domain	AAV1/SERCA2a	Placebo / Optimized
Symptomatic		
Quality of Life questionnaire	↑↑	↓
Functional		
6 Minute Walk Test	↔	↓↓
VO_2max	↔	↓↓
Biomarker	↔	↓↓
NT-proBNP		
Remodeling	↔	↓
Ejection Fraction		
End Systolic Volume	↑	↓↓

Fig. 2. Summary of the changes in heart failure domains seen after treatment with adeno-associated virus (AAV)1/sarco(endo)plasmic reticulum Ca^{2+}ATPase (SERCA2a) or placebo. Patients treated with placebo demonstrated evidence of worsening over the 12-month follow-up period. In contrast, the clinical course of patients who received high-dose AAV1/SERCA2a therapy appears to have stabilized or improved. NT-proBNP, N-terminal pro-B-type natriuretic peptide. Reproduced with permission from Jessup et al. [35].

with high-dose SERCA2a gene transfer therapy, however, was associated with an 88% ($p < 0.01$) risk reduction at the end of 1 year and an effect of almost equal magnitude at 81% has been maintained over the 3-year period of follow-up. A trend toward a reduction in mortality at the end of 3 years was also seen.

One important question regarding the use of SERCA2a gene transfer therapy in heart failure patients is whether there is evidence of persistent transgene expression in the myocardium. The availability of tissue from patients in CUPID who underwent cardiac transplantation or left ventricular assist device (LVAD) implantation provided an opportunity to assess whether this was the case. From the limited number of tissue samples available there is evidence that patients who were treated with high-dose SERCA2a gene transfer therapy who had low neutralizing antibody titers at the time of administration of the viral construct had evidence of transgene expression in the myocardium with the longest time from administration to sampling being 31 months [36]. Ongoing surveillance of the CUPID population should provide additional needed information about the longevity of transgene expression in treated patients.

Based on the encouraging results of CUPID, the pivotal CUPID 2 study was undertaken [38]. In CUPID 2, 250 advanced heart failure patients from sites in the USA and Europe were randomized to receive either high-dose AAV1/SERCA2a gene transfer therapy or placebo in a blinded fashion. Entry criteria for CUPID 2 were similar to those used in CUPID, except that the population was enriched to include patients at increased risk of events based on either recent heart failure hospitalization within the past 6 months or elevated levels of natriuretic peptide measured within 30 days of randomization. The study is event driven with a primary endpoint of 186 recurrent heart failure hospitalizations or terminal (death, cardiac transplantation or LVAD) events with patients required to be followed for at least 1 year in order to test the hypothesis that high-dose SERCA2a gene transfer will improve

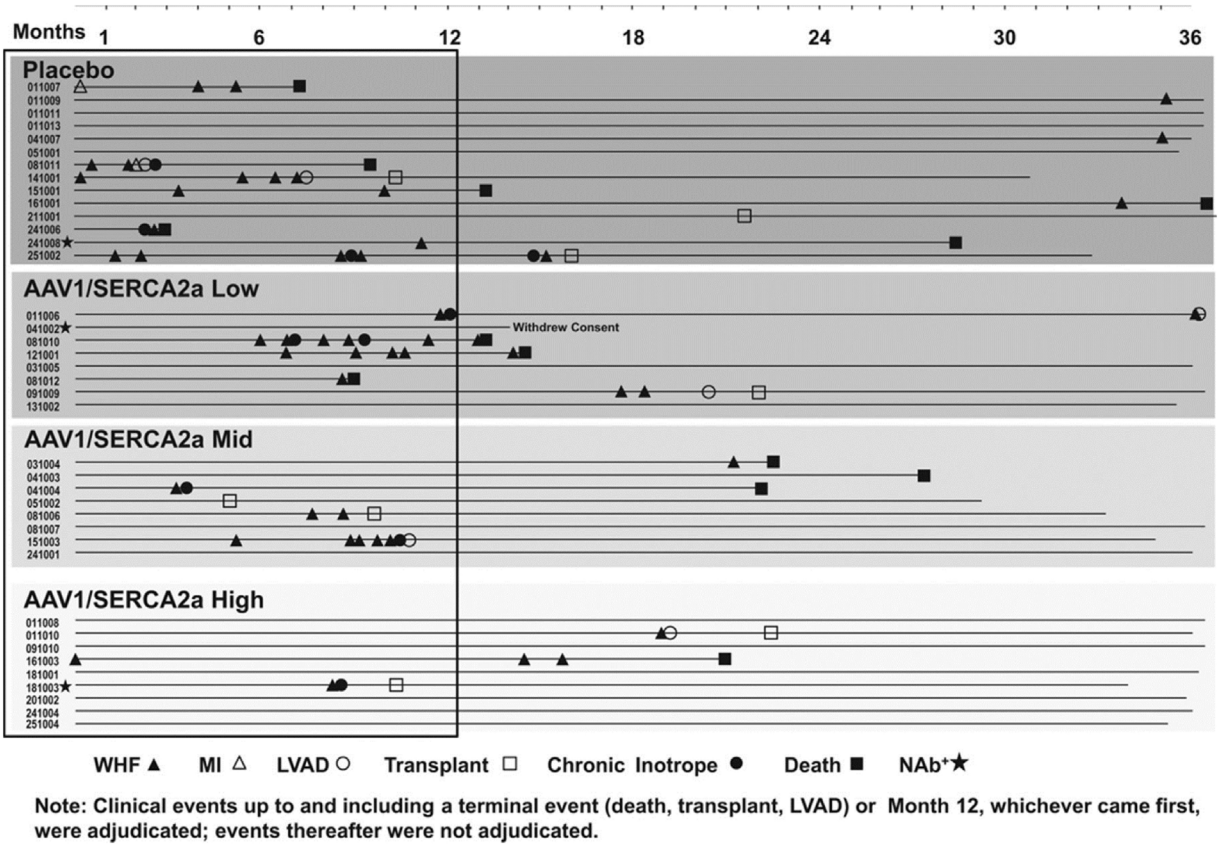

Fig. 3. Cardiovascular and Terminal Events in Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease phase 2. This figure represents the clinical course for study patients starting from the date of infusion of AAV1/SERCA2a or placebo; each line represents a single patient. A star at the beginning of a line represents a patient with anti-AAV1 NAb titer that was <1:2 during screening but ≥1:2 at baseline. AAV1/SERCA2a, recombinant adeno-associated viral vector (AAV) containing human sarcoplasmic reticulum Ca^{2+}ATPase 2a (SERCA2a) gene; LVAD, left ventricular assist device; MI, myocardial infarction; NAb^+, positive for anti-AAV1 neutralizing antibody (NAb) at baseline; WHF, worsening heart failure.
Reproduced with permission from Greenberg et al. [38].

outcomes in heart failure patients. The requisite number of primary events has been achieved in this study and the 1-year follow-up for all patients will be completed early in 2015.

Future directions

Although gene transfer therapy appears to be promising for treating heart failure, there are many issues that remain. The most immediate is in regards to the presence of neutralizing antibodies. AAV-related disease (which is mostly clinically silent or mild in nature) is common in the population and many people develop titers of neutralizing antibodies in response to these infections. The presence of these antibodies, however, appears to adversely affect the efficacy of treatment and it is currently recommended that only patients with titers of <1.2 be treated. In CUPID 2, in which serum samples were collected from over 1500 patients, 60% were excluded based on antibody titers. Although there was significant variability between countries, no specific patterns could be detected. Only patient age correlated with qualifying AAV1 neutralizing antibody titers. If these results are representative, then the presence of such antibody titers will be an important impediment to AAV-based gene transfer approaches. Studies aimed at reducing antibody titers either by medical therapy, plasma exchange, or other approaches will need to be carried out in order to make this therapy more widely available.

Despite the fact that currently available AAV vectors demonstrate high avidity for cardiac tissue, uptake in other organs and tissue throughout the body occurs raising the possibility of off-target effects of therapy that could be harmful. Thus, continued development of more cardiac-specific vectors is needed. Similarly, developing vectors that can deliver genetic material beyond the currently limited transgene size would be an important step forward. Constructs currently being developed for gene transfer therapy utilize strong promoters (e.g. the cytomegalovirus promoter is used to drive SERC2a) in order to maximize transgene expression. While this may be helpful initially, it is less certain whether continued high-level expression of the therapeutic gene will be helpful and could, potentially, be harmful. Therefore, systems in which transgene expression could be regulated according to prevailing conditions or need and which could be shut off if shown to be deleterious would be advantageous for future work in this area. Finally, defining new targets (e.g. receptors, signaling molecules, structural proteins or enzyme) that are amenable to modification by gene transfer techniques remains an important future consideration.

Disclosures

Dr Greenberg is a consultant for and receives honoraria from Celladon.

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